Application for consent to release a GMO

Part A2: Data or results from any previous releases of the GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

The GMOs that are the subject of this application have not been released before.

Part A3: Details of previous applications for release

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

There have been no previous applications to release these GMOs.

Part A4: Risk assessment and a statement on risk evaluation

Summary
Environmental risks

The overall risk of harm to the environment arising from this trial is assessed as extremely low.

Human health risks

The overall risk of harm to human health arising from this trial is assessed as extremely low.

Risk assessment
Conclusions on the Potential Environmental Impact from the Release or the Placing on the Market of GMOs

i. Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

The phenotype of the gene-edited and genetically modified barley lines in this field trial, including morphology, pollination, and seed-set do not appear to differ from non-transgneic barley cv. Golden Promise plants. We, therefore, expect no difference in the dissemination of pollen and seeds compared to non-transgneic barley cv. Golden Promise plants. The major trait in the barley lines is that their abilities in interaction with arbuscular mycorrhizal fungi (AMF) will be altred. Changes in the extent of mycorrhizal colonisation and soil mycorrhizal hyphae in genetically modified and gene-edited lines are not expected to have any adverse environmental effect over the time span of this trial or thereafter, and will not affect the subsequent ability of mycorrhizal interactions to form in these soils.

ii. Any selective advantage or disadvantage conferred to the GMHP.

It is not expected the gene-edited and genetically modified barley lines in this field trial have a growth chrachterstic that likely to confer a selective advantage or disadvantage. However, the overexpression of genetically modified lines (OxHvNSP2 and OxMtNSP2) by their abilities to override phosphate suppression of mycorrhizal colonisation can take advantage of the association with AMF even in the current highly phosphorus-rich soils in the UK.

iii. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.

Barley cv. Golden Promise is naturally an inbreeding self-pollinating crop with very low rates of cross-pollination with other barley plants. However, the pollination of Golden Promise by its wild relative *Hordeum bulbosum* through breeding in glasshouses can lead to haploid plants. There are no sexually compatible wild barley relatives present on the release site as no barley, other cereals or grasses will be cultivated or allowed to grow within 20 metres of the trial.

iv. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids and pathogens (if applicable).

The target organisms of this field trial are the naturally occurring arbuscular mycorrhizal fungi. The aim of this study is to evaluate the impact on biomass and yield following the use of commercial arbuscular mycorrhizal fungi (AMF) mixed

inoculum on the performance of the gene-edited and genetically modified barley cv. Golden Promise lines. The CRISPR mutant gene-edited lines used in this release have aborted or significantly reduced colonization by AMF. Conversely, the *NSP2* genetically modified overexpressing lines show higher mycorrhizal colonisation, even at high soil phosphorus concentrations which usually act to suppress mycorrhization. Plots containing *NSP2* overexpression lines (OxHvNSP2 and OxMtNSP2) may show a slightly increased extent of soil-borne mycorrhizal fungal mycelium, while plots containing the genetically edited line including *symrk-2*, *ccamk-1*, *ccamk-2*, *cyclops-2*, *cyclops-3*, *ram1-1*, *ram1-2*, *nsp1-1*, *nsp1-4*, *nsp2-2, and nsp2-4* will likely have reduced quantities of these fungi. Changes in the extent of mycorrhizal colonisation and soil mycorrhizal hyphae in genetically modified and gene-edited lines are not expected to have any adverse environmental effect over the time span of this trial or thereafter, and will not affect the subsequent ability of mycorrhizal interactions to form in these soils.

v. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

Barley interacts with a range of pests and fungal pathogens and may also interact with multiple fungi, bacteria, and protists in the rhizosphere. Mycorrhizal fungal colonisation has been demonstrated to reduce plant susceptibility to pests and pathogens. As a result of reduced colonisation by mycorrhizal fungi, gene-edited lines may show slightly increased susceptibility to foliar pathogens, while genetically modified lines are likely to show reduced susceptibility to pathogens. There are no adverse environmental effects predicted by changes to these interactions. Other interactions are not expected to be affected in any way by the traits carried by the plants.

vi. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into direct contact with, or in the vicinity of the GMHP release(s).

No toxic, allergenic, or harmful effects on human health are envisaged. The gene-edited and genetically modified barley have exhibited a difference in the expression pattern of a number of genes involved in the plant metabolites. None of these genes are known to be toxic or harmful to human health, nor are they known to exert any toxic or allergenic effects.

vii. Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed.

The gene-edited plant lines used in the field trial are considered to be transgene free and free of CAS9 editing machinery system. The gene edition in the symbiosis pathway genes does not lead to encoding any new proteins that are harmful to animal health. The genetically modified barley have exhibited a difference in the expression pattern of a number of genes involved in the plant metabolites. None of these genes are known to be toxic or harmful to human health, nor are they known to exert any toxic or allergenic effects. Any unknown hazards arising from the expression and ingestion of foreign proteins by domestic or farm animals will not be realised because the barley plants will not be used for animal feed. The site is enclosed and all care will be taken to ensure that no seed remains on the surface. Various bird-scaring devices will be used to keep birds out during the growing season.

viii. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

Biogeochemical processes are not expected to be affected by the cultivation of the genetically modified plants.

ix. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.

The site will be prepared according to standard agronomic practices for sping barley cultivation. The trial will receive standard farm practice as regard to herbicides, fungicides, nitrogen, sulphur and other fertilisers except some of the plots will not receive phosphorus fertiliser. The site will be monitored regularly throughout the trial. Due to the potential for minor increases in foliar pathogen susceptibility in the gene-edited lines, plots containing these lines will require additional monitoring and the application of approved fungicide(s) where appropriate. There are no expected adverse environmental effects. Harvest will occur by the end of September depending on weather conditions. The trial will be moved within the field year to year to prevent the plants being affected by pathogens. The plot will be left fallow after harvesting, monitored for the remainder of the year, and sprayed with non-selective herbicides. A sample of plants may be hand-harvested, conditioned, and threshed to supply seeds for

research purposes. All such small samples removed from the trial site will be stored in containment prior to use and will eventually be autoclaved before disposal. The site will be harvested by the plot combine. Grain that is not required for analysis or to provide seed for future trials will be disposed of by incineration, autoclaving, or deep burial at a local authority-approved landfill site using an approved contractor, while any material remaining after analysis will be autoclaved before disposal. All straw will be chopped and left on site. The combine will be cleaned prior to leaving the site so that all traces of plant material from the trial will remain in the trial area. All transport of material will be logged.

	Step1: ***Potential hazards which may be caused by the characteristics of the novel plant***	**Step 2:** ***Evaluation of how each hazard could be realised in the receiving environments***	**Step 3:** ***Evaluation of the magnitude of harm caused by each hazard if realised***	**Step 4:** ***Estimation of how likely/often each hazard will be realised as harm***	**Step 5:** ***Modification of management strategies to obtain lowest possible risks from the deliberate release***	**Step 6:** ***Overall estimate of risk of harm caused by the release for each hazard***
a	**Increased invasiveness in natural habitats or persistence in agricultural habitats.**	Increased invasiveness may arise from intended or unintended effects of the genetic modification that result in barley plants with a more 'weedy' habit that are better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	Barley is an annual species that requires active management to out-compete weedier plants. Left unmanaged, barley and survive in nature and thus has a low baseline of invasiveness and persistence. Even if intended or unintended effects of the genetic modification resulted in major changes in invasiveness or persistence, it is	It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness or persistence. If it were to occur, this hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and become established	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. there will be a barley pollen barrier of 3 metre surrounding the perimeter of the trial Surrounding the trial site is a 20 metre area in which no cereals or wild grasses will be allowed to grow.	Overall risk is negligible.

			considered that this would not result in significant environmental harm for agricultural or unmanaged ecosystems. Barley is a benign plant that can be easily managed by cultivation or herbicides. The magnitude of harm if the hazard were realised is, therefore, considered to be low.	elsewhere. This is very unlikely as barley pollen is relatively heavy so does not travel far, and it has a short half-life. Cereals and grasses will not be allowed to grow within 20 m of the trial site, and spontaneous crossing between barley and its closest wild relatives in the UK has not been observed. Seed removal from the site will be rigorously managed. The chances of modified barley plants establishing themselves outside the trial site are considered to be negligible.	Steel mesh security type fencing will be used to enclose the site to prevent animal access. Various bird-scaring devices will be used to keep birds out during the growing season.	

b	**Selective advantage: improved resistance to *P. infestans.***	None of the genes introduced confer characteristics that add intrinsic competitive abilities to improve resistance *to P. infestans.*	Negligible.	Very unlikely.		No risk.
c	**Selective advantage: improved resistance to potato cyst nematodes**	None of the genes introduced confer characteristics that add intrinsic competitive abilities to improve resistance to potato cyst nematodes.	Negligible.	Very unlikely.		No risk.
d	**Selective advantage: resistance to sulfonylureas and**	Such selectable markerd do not exist in the plants for this field trial. Beside,	Negligible. Plants containing the CSR selectable marker can be readily	Very unlikely.	Non-selective herbicide treatment will be used.	No risk

	imidazolinones provided by the selectable marker gene (*CSR*)	non-selective herbicide treatment will be used in the context of this field trial.	eliminated by other effective herbicides, such as glyphosate.			
e	**Selective advantage or disadvantage conferred to sexually compatible plant species**	Selective advantage or disadvantage may result from intended or unintended effects of the genetic modification. This hazard could be realised in the receiving environment via out-crossing to sexually-compatible species outside the trial site.	The traits of altered associations with AMF do not change morphology, pollination, and seed-set. Barley cv. Golden Promise is naturally an inbreeding self-pollinating crop with very low rates of cross-pollination with other barley plants. The magnitude of harm if the hazard were realised is, therefore, considered to be low.	This hazard would be realised only if pollen possessing genes encoding these traits were to spread from the trial site and become established. This is very unlikely as barley pollen is relatively heavy so does not travel long distances, and it has a short half-life. Cereals and grasses will not be allowed to grow within 20m of the trial site, and spontaneous crossing between wheat and its closest wild relatives in the	There will be a barley pollen barrier of 3 metre surrounding the perimeter of the trial. There are no sexually compatible wild barley relatives present on the release site as no barley, other cereals or grasses will be cultivated or allowed to grow within 20 metres of the trial from the outer edge of the pollen barrier. Steel mesh security type fencing will be used to enclose the site to prevent animal access.	Overall risk is extremely low.

				UK has not been observed. The likelihood of this hazard resulting in environmental harm is, therefore, considered to be extremely low.		
f	**Potential environmental impact due to interactions between the novel plant and target organisms**	The intended effect is to alter the association of GMHP to arbuscular mycorrhizal fungi (AMF) in low and high phusphrus concentrations in the soil. Therefore, the gene-edited lines may show a selective disadvantage of AMF colonization while the genetically modified overexpression lines by their abilities to override phosphate	Changes in the extent of mycorrhizal colonisation and soil mycorrhizal hyphae in genetically modified and gene-edited lines are not expected to have any adverse environmental effect over the time span of this trial or thereafter, and will not affect the subsequent ability of mycorrhizal interactions to form in these soils. Therefore, the	The target organisms of this field trial are the naturally occurring arbuscular mycorrhizal fungi. Plots containing *NSP2* overexpression lines may show a slightly increased extent of soil-borne mycorrhizal fungal mycelium, while plots containing the genetically edited line will likely have reduced quantities of these fungi. These	The commercial arbuscular mycorrhizal fungi (AMF) mixed inoculum will be sued. There will be two phosphorus treatments; application and no application of phosphorus fertiliser to evaluate the effect of AMF.	Overall risk is negligible. Potentially, there may be a positive impact on foliar microflora in some plots.

		suppression of mycorrhizal colonisation can take advantage of the association with AMF even in the phosphorus-rich soils. This could have an environmental impact if changes in interactions with AMF resulted in the plants being better able to thrive in the current fertiliser practices.	magnitude of the harm Overla risk is negligible	altered interactions are not harmful to the plants.		
g	**Potential environmental impact due to interactions between the novel plant and non-target organisms**	Changes in the plants' interactions with non-target organisms including a range of pests and fungal pathogens could result from the intended effects of the genetic modification. This	Mycorrhizal fungal colonisation has been demonstrated to reduce plant susceptibility to pests and pathogens. There are no adverse environmental effects predicted by	As a result of reduced colonisation by mycorrhizal fungi, gene-edited lines may show slightly increased susceptibility to foliar pathogens, while genetically modified lines are likely to	Due to the potential for minor increases in foliar pathogen susceptibility in the gene-edited lines, plots containing these lines will require additional monitoring and the application of	Overall risk is negligible.

		could have an environmental impact if changes in interactions with non-target organisms resulted in the plants being better able to thrive in uncultivated environments or to persist in agricultural habitats.	changes to these interactions. Other interactions are not expected to be affected in any way by the traits carried by the plants.	show reduced susceptibility to pathogens.	approved fungicide(s) where appropriate. There are no expected adverse environmental effects	
h	**Potential effect on human or animal health due to the introduced genes**	By contact or ingestion of GM plant material.	11 out of 13 lines in this field trial do not contain even any transgene. The genetic modification in all the lines are not expted to result in the synthesis of the products that are harmful to human health. The gene-edited and gene-modified barley have exhibited a	Some contact between the GM plants and humans is inevitable. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. However, it is extremely unlikely that they will ingest any plant material. It	No plant material from the trial will enter the food or animal feed chain. Any unexpected occurrences that could potentially result in adverse environmental effects or the possibility of adverse effects on human health will be notified to the GM	Overall risk is extremely low.

			difference in the expression pattern of a number of genes involved in the plant metabolites. None of these genes are known to be toxic or harmful to human health, nor are they known to exert any toxic or allergenic effects. Any unknown hazards with respect to human health arising from the expression and ingestion of foreign proteins will not be realised because the barley plants will not be consumed by humans.	is more likely that small mammals such as mice, invertebrates, and birds may come into contact and/or ingest plant material.	inspectorate immediately	
i	**Potential effects on biogeochemical processes**	No detrimental effect on the soil is	Soil fertility is not expected to be affected any	Any effect is expected to be comparable to that	Conventional agricultural practice, except some of the	Overall impact is negligible.

	(changes in soil decomposition of organic material)	expected from the introduced genes.	differently due to the cultivation of the genetically modified barley plants as compared to spring barley cv. Golden Promise	of non-genetically modified spring barley cv. Golden Promise under conventional agricultural practice.	plots, will not receive phosphorus fertiliser.	
j	**Possible environmental impact due to changes in cultivation practice**	No major differences in the cultivation and management of the GMHP will occur except some of the plots will not receive phosphorus fertiliser. The magnitude of any effects arising from changes in cultivation practice will be negligible.	The magnitude of any effects arising from changes in cultivation practice will be negligible.	The freuency that this hazard may be realised is low. The trial comprises only 108 x [1.5 x 4.25 metre] plots , and will be sown for only five growing seasons.	Conventional agricultural practice	Overall risk is extremely low.

Part A5: Assessment of commercial or confidentiality of information contained in this application.

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

The information provided in this application does not need to be kept confidential.

Part A6: Statement on whether detailed information on the description of the GMO and the purpose of release has been published
Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

The GMOs in the release have been described in the manuscript by Li et al. which has been submitted to the Journal *Cell* in December 2021. However, details of the proposed release and its purpose have not yet been published.